

Machine
Translation

JP1994324046A

1994-11-25

Bibliographic Fields

Document Identity

(19)[

日本国特許庁

(12)[

公開特許公報

(11)[

特開平

(43)[

平成 . 年 月 . . 日

Public Availability

(43)[

平成 . 年 月 . . 日

Technical

(54)[

悪性腫瘍・検出方法及・キ

(51)[

5 版 .

G01N 33/574 Z 8310-2J

33/53 D 8310-2J

[

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[

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Filing

[

未請求

(21)[

特願平

(22)[

平成 . 年 月 . . 日

Foreign Priority

(31)[

(19) [Publication Office]

Japan Patent Office (JP)

(12) [Kind of Document]

Unexamined Patent Publication (A)

(11) [Publication Number of Unexamined Application]

Japan Unexamined Patent Publication Hei 6 - 324046

(43) [Publication Date of Unexamined Application]

1994 (1994) November 25 days

(43) [Publication Date of Unexamined Application]

1994 (1994) November 25 days

(54) [Title of Invention]

DETECTION METHOD AND KIT OF MALIGNANT TUMOR

(51) [International Patent Classification, 5th Edition]

G01N 33/574 Z 8 31 0-2J

33/53 D 8 31 0-2J

[Number of Claims]

4

[Form of Application]

FD

[Number of Pages in Document]

7

[Request for Examination]

Unrequested

(21) [Application Number]

Japan Patent Application Hei 6 - 71308

(22) [Application Date]

1994 (1994) March 17 days

(31) [Priority Application Number]

JP1994324046A

1994-11-25

特願平

Japan Patent Application Hei 5 - 82506

(32)[

(32) [Priority Date]

平 月 . . 日

1993 (1993) March 18 days

(33)[

(33) [Priority Country]

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1994-11-25

JP1994324046A

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Abstract

(57)[

(57) [Abstract]

[

[Objective]

体液可溶性 E 量 ・ 測定す
 提 癌性腫瘍 ・ 検出す ・ 方法 ・ 及 ・ キ

Method of detecting malignant tumor by measuring body fluid solubility Ecadherin quantity. And kit is offered.

[

[Constitution]

体液中 ・ 体液可溶性 E 量 ・ 測定 ・
 そ
 法 ・

detection method " of malignant tumor which measures body fluid solubility Ecadherin quantity in body fluid, compares value with healthy value

体液可溶性 E 反応性 ・ 有す ・ 抗体
 ・ 含有す ・ 上記検出方法 ・ 実施す ・ た め
 性腫瘍検出用キ

malignant tumor assay kit " in order contains antibody which possesses reactivity in the body fluid solubility Ecadherin, to execute above-mentioned detection method

体液 ・ 例 ・ 血清 ・ 血漿 ・ 及 ・ 尿 ・

There is a blood serum " blood plasma " and a urine as example of body fluid.

[

[Effect(s)]

悪性腫瘍 ・ 検出 ・ 迅速 ・ 簡便 ・ 行 ・ き

It detects malignant tumor quickly and simply, it is possible .

Claims

[

[Claim(s)]

[1 ・

[Claim 1]

体液中 ・ 体液可溶性 E 量 ・ 測定 ・
 そ
 性腫瘍 ・ 検出方法 ・

body fluid solubility Ecadherin quantity in body fluid is measured, value is compared with healthy value detection method " of malignant tumor which is made feature

[2 ・

[Claim 2]

体液可溶性 E 反応性 ・ 有す ・ 抗体
 ・ 用 ・ 体液可溶性 E 量 ・ 測定す ・
 請求項 1 記載 ・ 方法 ・

method " which is stated in Claim 1 which measures body fluid solubility Ecadherin quantity making use of antibody which possesses reactivity in the body fluid solubility Ecadherin

[3 ・

[Claim 3]

体液 ・ 血清 ・ 血漿 ・ 又 ・ 尿 ・ 用 ・ 請求項
 1 又 ・ 2 記載 ・ 方法 ・

method " which is stated in Claim 1 or 2 which uses blood serum " blood plasma " or the urine as body fluid

[4 ・

[Claim 4]

体液中 ・ 体液可溶性 E 量 ・ 測定 ・
 そ
 瘍 ・ 検出 ・ 行 ・ た め
 溶性 E 反応性 ・ 有す ・ 抗体 ・ 含有
 ・ す 特徴 ・ す ・ 悪性腫瘍検出用キ

body fluid solubility Ecadherin quantity in body fluid is measured, with kit in order to detect malignant tumor value is compared with healthy value with, antibody which possesses reactivity in body fluid solubility Ecadherin is contained malignant tumor assay kit " which is made feature

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Specification

[

[0001]

[

・本発明・悪性腫瘍・検出方法及・検出用キ
・・関す・・

[0002]

[

・・・・・分子量約0 万前後・膜貫通・
・・持・細胞膜表面分子・・・・細胞間
・接着・関・・共存す・ Ca^{2+} ・・・依存的・
機能・発揮す・・・・・質・・・・・

現在・・・・・数・分子種・発見・
・・・・・そ・最・古・単離同定・・た
分子種・・E・・・・・N・・・・P・
・・・3種・・・・知・・・・・

・・・・3種・分子種・そ・・・・同一分子種
同志・・・・選択的・結合す・性質・示す・
・知・・・・・同質結合機構・
・・・族・特徴・1・・・考・

・・・・・分子種・・・・・特生命体・発
生・分化・過程・組織構築・働・重要・細胞
間接着分子・・・・・知・・・・・神経組
織・心臓・胎盤・・・・・体内・特定・臓器
・・・・・一定・分子種・主要・分布・
・・・・多・・・・・組織・形成・細胞接
着機能・発揮す・・・・・考・・・・・M・
・・・・(M.Takeichi)・・・・・(Science)・第251
巻・第1451-1455頁(1991)・

・・・代表的3種・・・・・E・
・・・・・上皮細胞・上皮
組織・主要・発見す・分子・・・・免疫組織染
色法・・・・用・・・・疾患・組織中・局在
・・・・調・・・・・Y・
(Y.Shimoyama)・・・・・(Cancer
Research)・第49巻・第2128-2133頁(1989)・

そ・・・・検討・結果・・・・特・悪性腫瘍組織
・・・・E・・・・・量・減少・・・・現
象・数多・見出・

・た・動物実験・・・・・悪性腫瘍細胞株・
・・・E・・・・・量・多・細胞間接着・強
・株・比・・・・・量・少・細胞
間接着・弱・株・方・悪性度・高・高・破

[Description of the Invention]

[0001]

[Field of Industrial Application]

this invention regards detection method and assay kit of
malignant tumor.

[0002]

[Prior Art]

cadherin with plasma membrane surface molecule which has
membrane penetration domain approximately of molecular
weight approximately 100,000, is protein which
shows function in Ca^{2+} ion dependent which coexists in regard
to glueing between the cell-cell.

To presently molecular type is discovered by cadherin many,
3 kinds of the Ecadherin *Ncadherin *Pcadherin are well
known even among those to be oldest as molecular type
which isolation and identification is done.

As for molecular type of these 3 kinds only respective same
molecule kind the selectively shows property which is
connected, it is known, these same material coupling
mechanism are thought one of feature of cadherin family.

These cadherin molecular type are important intercellular
adhesion molecule which with process of occurrence and
differentiation of especially living thing works in organization
construction, it is known, in specific organ of inside the body
such as nerve organization and heart and placenta respective
fixed molecular type distribution has made principal, is
many, It is thought that cell adhesion function is shown in
formation of the respective organization, {M. bamboo I jp8
(M.Takeichi), Science (Science), second Vol.51 * 14th
51~1455 page (1991)}.

Among cadherin of these representative 3 kinds, as for
Ecadherin with molecule which is revealed principally in
epithelial cell and epithelial tissue in human and mouse etc,
localized in organization of various disease such as
is inspected making use of immunohistological staining
method etc, {Y. ti haze * (Y.Shimoyama) and others,
Cancer Research (0008 - 5472, CNREA8) (Cancer
research), Vol.49 * second 128-2133 page (1989)}.

phenomena which Ecadherin amount of expression has
decreased from result of those examination, in especially
malignant tumor organization is many discovered.

In addition, regarding animal experiment, tendency which
forms transfer lesion with probability where Ecadherin
amount of expression to be less strain where intercellular
adhesion is weak degree of malignancy is higher intercellular

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率・転移病巣・形成す傾向・見出・
 U.H. (U.H.Frixen) (Journal of Cell Biology)
 第113巻・第173-185頁(1991)

結果・細胞表面・表示
 E・量・悪性腫瘍転移性能・悪性
 度・間・密接・関連・推測

[0003]

た一方・本質的・膜貫通領域・分子内
 部・有す・界面活性剤・含
 溶媒中・可溶化す・性・持
 た・界面活性剤・含・溶媒中・細胞
 抽出液・分子・胞外
 領域・一部・極め・切断
 ・易・切・出・た・分子量8万前後・低分子
 ・解断片・界面活性剤・含
 ・水系溶媒中・可溶化状態・存在・き
 既・知・M.J.
 (M.J. Wheelock) (Journal of Cellular
 Biochemistry) 第34巻・第187-202頁(1987)

生
 分解物・動物・生体液中・存在す
 従来全知・た
 た・従来・解物・主要
 構成分子・す・可溶性・溶媒
 ・測定・含有量算出す・技術・存在
 ・た・起因す

[0004]

【解決・課題・】

た古・解析・きた腫瘍・E
 ・発見量・悪性腫瘍・疾患・関連
 ・従来E・検出技術・用
 ・きた抗体・組織化学的検出法
 ・法・免疫
 沈降法・方法・定性的方法
 ・患者・組織生検材料
 採取・危険・伴・た・試薬・機器・準備
 ・時間・点・実際・疾患
 ・診断・臨床の応用・極め・難
 ・た

本発明・目的・体液可溶性E・簡便

adhesion is strong inside Ecadherin amount of expression of
 malignant tumor cell line to be many in comparison with
 strain where, is high seesand comes out and is {U.H.
 * jp9 habit (U.H.Frixen) and others, journal * of * cell
 biology (Journal of Cell (0092 - 8674) biology), Vol.113 *
 17th 3-185 page (1991)}.

From these results, it is presumed in quantity of Ecadherin
 which is been indicator in in cell surface and transition talent
 of malignant tumor and between degree of malignancy that
 there is relation of intimate.

[0003]

Portion of extracellular domain of cadherin molecule it is easy
 to be cut off with the quite protease in addition on other hand,
 as for cadherin which essentially possesses membrane
 spanning region in intramolecular section with property which
 solubilizing is done only in in solvent which includes
 the boundary surfactant etc, in addition, in cell extracted liquid
 etc in in solvent which does not include boundary surfactant,
 As for low molecular weight cadherin disassembly fragment
 approximately of molecular weight 80,000 which is quarried
 out in aqueous solvent which does not include boundary
 surfactant etc it can exist it is already known with solubilizing
 state, {M.J. fee lock (M.J. Wheelock) and others, journal *
 of * cellular * biochemistry (Journal of Cell (0092 - 8674) uar
 Biochemistry (0006 - 2960, BICHA W)), Vol.34 * 18th
 7-202 page (1987)}.

But, it was not completely informed until recently that
 cadherin lysate which it occurs by protease exists in human
 and animal or other organism liquid.

In addition, this until recently measures solution medium
 concentration of solubility cadherin which designates cadherin
 lysate as principal constituent molecule, originates in also the
 technology which calculates content not existing.

[0004]

[Problems to be Solved by the Invention]

In addition, for a long time concerning relation between
 Ecadherin amount of expression and malignant tumor or other
 disease on cell which is analyzed with antibody which is
 used until recently as Ecadherin detection technology as for
 histochemical detection method and flow site * tri- and
 Western blot method and immunoprecipitation or other
 method which with qualitative method with thing, in recovery
 of organization biopsy material from patient etc hazard
 accompanying, Those whose diagnosis or other clinical
 application of disease quite is difficult actually at or other
 point where time is required for reagent and the preparation
 etc of equipment.

It is to offer method and kit where objective of this invention

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・ 定量す ・ 方法確立 ・ 該方法 ・ 用 ・ 悪性
腫瘍 ・ 検出す ・ 方法及 ・ キ
・ 提供す

[0005]

【課題・解決す ・ た め手段 ・

本発明 ・ 概説す ・ ・ ・ 本発明 ・ 第1 ・ 発明 ・
悪性腫瘍 ・ 検出方法 ・ 関 ・ ・ 体液可溶性 ・ ・
・ ・ ・ 量 ・ 測定 ・ ・ ・ そ
・ ・ 特徴 ・ す ・ ・

・ た 本発明 ・ 第2 ・ 発明 ・ 悪性腫瘍検出用キ
・ ・ ・ 関 ・ ・ 体液中 ・ 体液可溶性 ・ ・ ・ ・ 量
・ 測定 ・ ・ ・ そ
・ 悪性腫瘍 ・ 検出 ・ 行 ・ た め
体液可溶性 E ・ ・ ・ ・ ・ 反応性 ・ 有す ・ 抗体
・ 含有す ・ ・ ・ ・ 特徴 ・ す ・ ・

[0006]

本発明者 ・ ・ 以上述 ・ ・ ・ た 従来技術 問題
点 ・ 考慮 ・ ・ 体液可溶性 E ・ ・ ・ ・ 溶液中
微量濃度 ・ 簡便 ・ 測定す ・ 方法確立 ・ ・ そ
測定法 ・ ・ ・ ・ ・ 及 ・ 動物 ・ 体液中 ・ 体液可溶
性 E ・ ・ ・ ・ ・ 一定量存在 ・ ・ ・ ・ ・ 見出
・ ・ ・ そ E ・ ・ ・ ・ ・ 量宿主 ・ 疾
患 ・ 特 ・ 悪性腫瘍 ・ ・ ・ ・ 有意 ・ 上昇す 現
象 ・ 見出 ・ ・ 本発明完成す ・ 至 ・ た ・

・ た ・ 本発明 ・ 方法 ・ 用 ・ ・ た 試薬類 ・ ・
・ め 化 ・ ・ ・ ・ 簡便 ・ 測定 ・ 簡 ・ ・
た ・

[0007]

本発明 ・ ・ ・ ・ 測定対象 ・ ・ ・ 体液可溶性 ・ ・
・ ・ ・ ・ ・ 可溶性 ・ 体液中存在す E ・ ・ ・
・ ・ ・ ・ 分解物 ・ ・ ・ ・ 非分解物 ・ ・ E ・ ・ ・
・ ・ 分子 ・ 由来す ・ す ・ ・ 分子 ・ 含 ・ ・ そ
・ ・ 主 ・ 分子量 8 万前後 E ・ ・ ・ ・ 解物
・ ・ ・ 構成 ・ ・ ・ ・

[0008]

本発明 ・ ・ ・ ・ 体液可溶性 E ・ ・ ・ ・ ・ 測定
方法 ・ ・ ・ ・ ・ 例 ・ 体液可溶性 E ・ ・ ・ ・ ・
反応性 ・ 有す ・ 抗体 ・ 用 ・ ・ 方法挙 ・ ・ ・
・ ・

該方法 ・ 例 ・ ・ ・ ・ ・ ・ ・ ・ ・ ・ ・
(EIA) ・ ・ ・ ・ ・ ・ ・ ・ ・ ・ ・
・ ・ ・ 免疫比濁法 ・ ・ ・ ・ ・ 凝集法 ・ ・ 体液可
溶性 E ・ ・ ・ ・ ・ 測定 ・ き ・ 方法 ・ ・ 何
・ ・ ・ ・ ・ 操作 ・ 簡便 ・ ・ ・ ・ ・ 特 EIA
・ 望 ・ ・ ・ ・

body fluid solubility Ecadherin simply establishes method
which quantification is done, detects malignant tumor making
use of said method.

[0005]

[Means to Solve the Problems]

If this invention is outlined, first invention of this invention
regards the detection method of malignant tumor, it measures
body fluid solubility Ecadherin quantity, value it is compared
with healthy value makes feature.

In addition second invention of this invention regards
malignant tumor assay kit, it measures body fluid solubility
Ecadherin quantity in body fluid, with kit in order to detect
malignant tumor value is compared with healthy value
with, antibody which possesses reactivity in body fluid
solubility Ecadherin it is contained it makes feature.

[0006]

As for these inventors above problem of Prior Art which is
expressed is considered, method which trace amount
concentration in solution of body fluid solubility Ecadherin
measures simply is established, body fluid solubility
Ecadherin constant amount exists in the body fluid of human
and animal with measurement method, phenomena which
you discover, body fluid solubility Ecadherin quantity rises
significantly in disease especially malignant tumor of host
index, this invention it reached to completion.

In addition, collecting reagent in order to use for method of
the this invention, to kit it converted, made simpler
measurement possible.

[0007]

Regarding to this invention, body fluid solubility Ecadherin
which becomes measurement subject, including all molecule
which such as lysate in Ecadherin molecule or nondegradable
ones of Ecadherin which with solubility exists in body fluid
derive, as for those mainly configuration it is done by
Ecadherin lysate approximately of molecular weight 8 0,000.

[0008]

You can list method which uses antibody which possesses
reactivity in for example body fluid solubility Ecadherin as
assay of body fluid solubility Ecadherin in this invention.

If enzyme immunoassay (EIA), it is a method which such as
radioimmunoassay ・ fluoro-immunoassay ・ immunity
turbidimetric method ・ latex coagulation method body fluid
solubility Ecadherin can measure as example of said method,
it is good anything, but the especially EIA is desirable from
fact that operation is simple.

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[0009]

体液可溶性 E 反応性・有す・抗体
 抗体又
 ・抗体 た 抗体由来動物
 一般・実験動物
 ・用 動物 使用
 抗原・対す・標的の反応性・確実
 大量製造・容易 由来
 抗体・使用す 特望

. 由来 抗体・例
 HEC D-1 (宝酒造社製)・SHE78-7 (宝酒造社製)
 た 顧品 本発明者・新た 作
 製・た SHE13-6・SHE3-10・挙

. 4 種 抗体・E
 分子上・互・異・部位・認識す 任
 意・組合 EIA 系・構築す き

例 HEC D-1 SHE3-10 組合
 SHE78-7 SHE3-10 組合 EIA 系・構築
 す き

・た SHE13-6・体液可溶性 E 対
 ・強・反応性・示 細胞上・非断片化 E
 弱・反応性・示

・た SHE13-6・使用す
 均一・体液試料中・体液可溶性 E
 測定す 簡

. 抗体・当業者 公知・技術
 ・適宜・断片化・標識化・固定化・修飾化
 ・処理・施

[0010]

本発明・使用す・体液 血清・血漿
 尿・髄液・羊水・腹水 液 通常・体
 外診断医療 採取 試料す
 含・組織・細胞・溶媒 洗浄液
 抽出液・含 す 特頻繁・用
 ・採取・簡便 血清・尿・好

[0011]

本発明者 体液可溶性 E 測定法
 EIA 体液可溶性 E
 反応性・有す 2 種・異
 抗体・一方

It is good with whichever of polyclonal antibody or monoclonal antibody as antibody which possesses reactivity in body fluid solubility Ecadherin, in addition antibody derivative animal mouse・rat・rabbit etc generally it is possible to use inside whichever of animal which is used as experimental animal, but specific reactivity for antigen being secure, uses mouse derivative monoclonal antibody from fact that large scale production is easy especially is desirable.

HEC D-1 (Takara Shuzo Co. Ltd. (DB 69-053-7063) supplied), SHE78-7 (Takara Shuzo Co. Ltd. (DB 69-053-7063) supplied) with other than commercial product which was said, you can list SHE1 3- 6・SHE3- 10 which these inventors produces anew as example of the mouse derivative monoclonal antibody.

Because monoclonal antibody of these 4 kinds recognizes site where top of Ecadherin molecule differs mutually, EIA system can be constructed with combination of option.

EIA system can be constructed with combination of combination, the SHE78-7 and SHE3- 10 of for example HEC D-1 and SHE3- 10.

In addition, SHE1 3- 6 it shows strong reactivity vis-a-vis body fluid solubility Ecadherin, only weak reactivity shows in non- fragmentation Ecadherin on cell.

Therefore, it depends on using SHE1 3- 6, from body fluid solubility Ecadherin in body fluid specimen is measured is possible in uniform.

As for these antibody fragmentation・labelling・fixation and decoration conversion or other treatment may be administered appropriately by known technology in person skilled in the art.

[0010]

As body fluid which is used for this invention, including specimen everything which can recover in conventional outside the body diagnosis medical care such as blood serum・blood plasma・urine・spinal fluid・sheep water and spleen・phosphorus pas liquid, also washing liquid and extracted liquid include with such as organization and solvent of cell it does, but it is used by especially frequent and blood serum and urine are desirable from fact that recovery is simple.

[0011]

As for these inventors, sandwich type EIA, it succeeded in to adsorb into microplate or other solid phase, labelling doing other with peroxidase enzyme, establishing one side of, monoclonal antibody where 2 kinds which possess reactivity

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固相・吸着・他方・・・キ・・・酵素・
標識・・・両者・組合・・・初め 確
立す・・・種・た

・・・体液可溶性E・・・測定IA法・・・数
ng/ml・・・低・濃度・溶液中・体液可溶性E
・・・定量・能・・・従来・・・種・
体液可溶性E・・・測定法・種・

・・・た・・・本発明者・・・体液可溶性E・・・
測定EIA・・・用・・・健康・成人・血清・血
漿・及・尿中・数ng/ml・・・数μg/ml・範囲・
体液可溶性E・・・検出・・・見
出・た

従来・・・又・動物由来・・・種・体液中・体
液可溶性E・・・存在す・・・事実・
見出・た

[0012]

更・・・本発明者・・・発明・発展・・・
数十名・疾患患者・体液中・体液可溶性E・
・・・量・分・数十名・健康人体液中・
体液可溶性E・・・量・分・比較・統
計的・解析・・・悪性腫瘍患者・・・有意・そ
・値・上昇す・現象・見出・腫瘍・・・
・病氣・診断・病状・把握・・・極め
用・・・見出・た

そ 試料・・・用・・・キ
・・・示す 共・更・採取・簡便・尿・体
液試料・・・用・・・可能・・・示
た

・・・様・体液可溶性E・・・簡便・体液中・
存在す・腫瘍・・・用・た 観例
過去・・・

[0013]

・・・た・・・本発明・方法・用・固相化抗体液・標
識抗体液・標準品溶液・基質溶液・・・試薬
類・・・め
簡便・体液可溶性E・・・測定・行・・・
き

・・・キ 試薬・溶液状・・・凍
結乾燥品・・・

[0014]

以上詳細・説明・た・・・本発明・・・従来
不可能・・・た 体液中・体液可溶性・・・
・微量測定・可能・・・血清尿・・・た
一般臨床検査・用・・・体液・使用・・・従

in body fluid solubility Ecadherin differ for first time by union
doing both as body fluid solubility Ecadherin measurement
method.

As for this body fluid solubility Ecadherin measurement EIA
method, you call several ng/ml, quantification of body fluid
solubility Ecadherin in solution of low concentration being
possible, body fluid solubility Ecadherin measurement method
of this kind is not reported until recently.

In addition, as for these inventors in blood serum・blood
plasma・of healthy human adult and in the urine from
several ng/ml body fluid solubility Ecadherin can be detected
in range of the several μg/ml making use of this body fluid
solubility Ecadherin measurement EIA, you discovered.

Until recently, fact that was not discovered body fluid
solubility Ecadherin exists in body fluid of this kind of human
or animal derived.

[0012]

Furthermore, these inventors these inventions developing,
compares distribution of body fluid solubility Ecadherin
quantity in body fluid of disease patient of several tens name,
and the distribution of body fluid solubility Ecadherin
quantity in healthy person body fluid of several tens name
analyzes statistically, phenomena where value rises
significantly in the malignant tumor patient index, Quite it is
useful in diagnosis of disease and grasp etc of the disease
condition as tumor marker, you discovered.

As and, blood serum you can use it shows, as body fluid
specimen furthermore also it is possible to use urine whose
recovery is simple as body fluid specimen it showed.

This way as for reported example which uses body fluid
solubility Ecadherin as tumor marker which exists in simple
body fluid there is not a past.

[0013]

In addition, solidification antibody liquid and labelled
antibody liquid which are used for method of this invention,
collecting standard article solution・substrate solution or
other reagent, compared to it measure simply body fluid
solubility Ecadherin by making kit, it is possible.

Furthermore, reagent which is included to kit is good
even with solution state and it is good even with lyophilized
product.

[0014]

As above explained in detail, until recently trace amount
measurement of the body fluid solubility Ecadherin in body
fluid which is impossible became possible depending upon
the this invention, using body fluid which is used for general

JP1994324046A

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来存在・新・規・腫瘍・疾患・
 ・・・・・可能・た・

[0015]

[

本発明・実施例・更・詳細・説明す・
 ・・本発明・実施例・限定・
 ・・・・・

[0016]

実施例 1

(1)免疫原・調製

健康妊婦・分・時・採取・胎盤・
 20mM・生理緩衝食塩水(150mM NaCl・
 5mM CaCl₂・2mM NEM・0.3mM PMSF・0.2%
 NaN₃・含・pH7.6)(以下 TBS・略す)・5
 回洗浄・付着・血液・除去・た・

洗浄・胎盤 500g・TBS1・共・
 ・・・・中・細片化・た・

充分・細片化・た後・高速遠心分離・抽
 出液上清・不溶物・分離・た(2000g×30分
 間)・

次・抗・E・
 抗体 HEC D-1(宝酒造社製)10mg
 活性化・(・社製)2g・吸
 着・HECD-1 抗体固定化・調
 製・た・

調製・た HEC D-1 抗体・充
 ・・・・TBS・洗浄・た・

引続き 先調製・胎盤抽出上清液・本・
 ・流・その後 TBS1・十分・
 ・洗浄・た・

・・・・洗浄・た後・8M 尿素・含有す TBS
 ・・抗体・吸着・た 撮物質・溶出・
 た・

溶出・た・質溶液・透析・入
 ・・1 晩 4 deg C 条件下・TBS5・中
 ・透析・た・

透析・完全・尿素・除去・た後・溶
 出・溶液・再・前記・HECD-1 抗体固
 定化・流・前記同様・操作・抗
 体・抗原物質・溶出・た・

溶出液・(・社製)・
 ・・・・質濃度 1mg/ml 程度・濃縮・2mM

laboratory test such as the blood serum and urine monitor of
 disease became possible with the novel tumor marker which
 does not exist until recently.

[0015]

[Working Example(s)]

this invention furthermore is explained in detail with Working
 Example, but the this invention is not something which is
 limited in these Working Example.

[0016]

Working Example 1

Manufacturing (1) immunogen

From healthy pregnant woman when giving birth placenta
 whichrecovers, 5 times was washed with 20 mM tris menses
 buff saline (150 mM NaCl・5 mM Ca Cl₂・2 mM NEM・0.3
 mM PMSF・0.2% NaN₃ are included, pH 7.6) (Below TBS
 you abbreviate.) and the blood which has deposited was
 removed.

placenta 500g which you washed with TBS1 liter flaking was
 done in the homogenizer.

flaking after doing, extracted liquid supernatant and insoluble
 matter were separated into the satisfactory due to high speed
 centrifugal separation (2000 gX 3 0 min).

Next, anti-human Ecadherin mouse monoclonal antibody
 HEC D-1 (Takara Shuzo Co. Ltd. (DB 69-053-7063)
 supplied) 10 mg cyanogen bromide activated Sepharose
 (Pharmacia make) adsorbinginto 2 g, it manufactured HEC
 D-1 antibody fixation Sepharose.

Filling up HEC D-1 antibody gel which it manufactures in
 glass column, TBS being you washed column well.

Continuously, placenta extraction supernatant liquid which is
 manufactured firstwas let flow to this column, after that with
 TBS1 liter column waswashed in fully.

After washing column, antigen substance which adsorbs into
 antibody column with TBS which contains 8 Murea was
 liquated.

Inserting protein solution which is liquated in dialysis tube,
 under overnight 4 deg Ccondition dialysis it did in TBS5 liter.

After removing urea completely with dialysis , this liquation
 protein solution was let flow to aforementioned HEC D-1
 antibody fixation column again, antigen substance was
 liquated from antibody column with operation of beingsimilar
 to description above.

It concentrated eluate in protein concentration 1 mg/ml extent
 with collodion pack (Sartorius supplied), dialysis itdid under

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CaCl₂・含・生理食塩水5・・・対・1晩
4 deg C 条件下・・・透析処理・た・

得・た・・・質溶液・・・抗・E・・・
・・・抗体・・・HECD-1・強・
反応す・抗原物質・主要・含・・・該抗原
物質・主・・・E・・・解物・・・構
成・・・(以後・・・質抗原・標準 E
・・・称す)・・・

・・・標準 E・・・500 μg・・・
・・・分注・・・80 deg C 以下・条件・免疫原・・・
・・・標準物質・・・使用す・時・・・凍結保存・
た・

[0017]

(2)・・・抗体・作製

実施例 1-(1)・取得・・・標準 E・・・500
μg・・・
・・・社製)・等量混合・6 週令 Balb/c・・・5
匹(日本・・・社製)・腹腔内・等分・投与・
た・

4 週間後・標準 E・・・500 μg・・・
・・・TDM(・・・社製)・等量混合・既免疫
・・・5 匹・再・等分・腹腔内投与・た・

更・4 週間後後者・投与操作・再度同・・・5
匹・実施・た・

そ 3 日後・・・そ・・・2 匹・・・脾
臓・摘出・・・方法・
従・・・細胞融合・行・・・細胞・
作製・た・

標準 E・・・鼠・・・使用・ELISA 法
・・・標準 E・・・反応性・有す・・・
・・・抗体・産生す・・・選択
た・

す・・・標準 E・・・TBS 中・・・希釈・
・・・終濃度 10 μg/ml・・・溶液 調製 96 穴・
・・・(・・・社)・適量分注・・・そ・
・・・4 deg C・・・24 時間静置・た・
中・溶液・す・廃棄 1%・血清・・・
・・・含・・・酸緩衝生理食塩水(以下 BSA/PBS
・称す)・・・満た 37 deg C 条件下
・・・1 時間静置・た・

そ 後・・・中・溶液・す・廃棄・・・
酸緩衝生理食塩水(以下 PBS・称す)・・・2 回
・・・洗浄・・・上記・・・作製・た・す
・・・細胞・培養上清・そ・・・
・・・各穴・添加・た・

overnight 4 deg C condition vis-a-vis physiological saline 5
liter which includes 2 mM Ca Cl₂.

protein solution which it acquires includes antigen substance
which reacts to the HEC D-1 which is a anti-human Ecadherin
mouse monoclonal antibody strongly in principal, said antigen
substance configuration is done by human Ecadherin lysate
mainly, (From now on, this protein antigen, is named standard
Ecadherin.).

In 500;μg at a time aliquot it did this standard Ecadherin in
tube, when - using with condition of 80 deg C or less to as
immunogen or the standard substance, freezing it retained.

[0017]

Production of (2) monoclonal antibody

Freund・Kong pre・jp7・adjuvant (Difco make) with
equivalent it mixed standard Ecadherin 500;μg which
is acquired with Working Example 1- (1), 6 -week-old
Balb/c mouse 5 animals equal parts did in intraperitoneal of
the (Clea Japan Inc. (DB 69-073-1062) supplied) and
prescribed.

jp9 via・・・bunt TDM (jp9・supplied) with equivalent it
mixed standard Ecadherin 500;μg 4 weeks, later equal parts
made again previous immune mouse 5 animals and the
intraperitoneal administration did.

Furthermore dosage operation of the latter of 4 weeks later
was executed for second time in same mouse 5 animals.

After 3 days, avulsion it did spleen from mouse of 2 animals
among those, followed to method of Kellar and Milstein and
and others did cell fusion, produced hybridoma cell.

You used standard Ecadherin as antigen, you selected
hybridoma which produces monoclonal antibody which
possesses reactivity in standard Ecadherin with ELISA
method.

Diluting namely, standard Ecadherin in TBS, it manufactured
solution which becomes final concentration 10 ;μg/ml, 96
-hole microplate suitable amount aliquot did in (jp10・・・
corporation), after 24 hours standing doing that way with 4
deg C, it abolished solution in the plate entirely, it filled up
phosphate buffered saline (It names below BSA/PBS.) which
includes 1% bovine blood serum albumin in plate, 1 hour
standing did under 37 deg C condition.

After that, as though solution in plate is entirely abolished and
twice plate is washed with phosphate buffered saline (It
names below PBS.), it is a description above, the culture
supernatant of all hybridoma cell which are produced was
added to each hole of the respective plate.

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そ 室温 1時間 静置 した後
PBS 3回洗浄 した

次 抗体 IgG キ 標識抗体液 (社製) BSA/PBS 適量 希釈 した 溶液 各穴 適量 添加 した

再 室温 1時間 静置 した後 PBS 3回 洗浄 残液 十分 取除 した

ABTS 基質液 (社製) 使用説明書 従 調製 洗浄終了後 各穴 適量 添加 室温 15分間静置 した

その後 96穴 (社製) 各穴 発色 波長 410nm 吸光度 算出 比較 強 発色 得 細胞株 選択 した

株 標準 E 弱 抗原抗体反応 起す 抗体 産す 株細胞 19株 選別 した

19株 細胞 対 2回 限界希釈 実施 再 上記 ELISA 法 行 最 適切 細胞 株 2株 最終 選択 細胞 産生す 抗体 抗体 抗体 SHE13-6 SHE3-10 命名 した

SHE13-6 SHE3-10 HEC D-1 SHE78-7 同様 E 産す 既 知 皮膚 細胞 A431 表面 強 反応す 間接酵素抗体染色法 確認 した

A431細胞表面 生理食塩水等 十分 洗浄 残留 可溶性 細胞表面 除去 した後 抗体 細胞表面 E 反応 間接酵素抗体染色法 調査 した SHE13-6 A431細胞表面 弱 反応 示 確め した

[0018]

そ SHE13-6 SHE3-10 HEC D-1 SHE78-7 同様 E 産す N 産す 知 HeLa細胞表面 全 反応 同様 間接酵素抗体染色法 確認 した

更 SHE13-6 SHE3-10 標準 E 適当量 SDS 電気泳動法

After standing doing 1 hour plate that way with room temperature, thrice you washed with PBS.

solution which dilutes anti-mouse IgG peroxidase labelled antibody liquid (Cappel supplied) next suitably with the BSA/PBS at a time suitable amount was added to each hole of plate.

Again, after standing doing 1 hour plate that way with room temperature, the thrice plate was washed with PBS, residual liquid was removed to fully.

Following ABTS substrate liquid (Amersham supplied) to use description, it manufactured, at a time suitable amount added to each hole of plate after washing ending, 15 min standing did with room temperature.

After that, you applied you calculated as absorbance of wavelength 410 nm, you selected these 96-hole microplate on plate reader (furo supplied), coloration of each hole hybridoma cell line where strong coloration is acquired by comparison with the background.

19 strain were sorted to this way, with standard Ecadherin as antigen as the strain cell which produces antibody which causes strong antibody-antigen reaction.

It executed limiting dilution cloning of twice vis-a-vis hybridoma cell of these 19 strain, did screening again with above-mentioned ELISA method, final selected most appropriate hybridoma cell clone strain 2 strain, monoclonal antibody which these cell produce SHE13-6 SHE3-10 designated respectively.

In same way as HEC D-1 SHE78-7 Ecadherin is produced strongly reacts to human skin cancer cell A431 surface which is already informed verified SHE13-6 SHE3-10, by indirect enzyme antibody dye method.

But, you wash A431 cell surface in fully, with such as physiological saline after removing solubility Ecadherin which has remained from cell surface layer when reaction to cell surface Ecadherin of these antibody was investigated with indirect enzyme antibody dye method, only weak reaction you show SHE13-6 in A431 cell surface, it was verified.

[0018]

And, SHE13-6 SHE3-10 did not produce Ecadherin in same way as HEC D-1 SHE78-7 and Ncadherin is produced completely does not react to HeLa cell surface which is informed verified by after all similar indirect enzyme antibody dye method.

Furthermore, SHE13-6 SHE3-10 separated reacts to protein of molecular weight approximately 80,000 verified

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.....分離.....膜電気的転写・た物・用・た.....法.....
HECD-1・SHE78-7・同様・分子量約8万・
.....質・反応す.....確認・た.....

[0019]

・た.....族・共通す・性質.....
.....発現.....培養細胞.....
.....質分解酵素.....分散.....際.....
・2-10mM.....共存下.....
.....分解.....そ.....分子・形・保.....
・残存す.....2-10mM EDTA 共存下.....
.....不在条件下.....分
解.....分子・消失す.....現象・確
認.....M.....(M.Takeichi).....
.....(Developmental
Biology)・第87巻・第340-350頁(1981)・

SHE13-6・10mM CaCl_2 ・含・0.02%.....
・溶液.....(社製)処理 A431 細胞抽出液中・
分子量約10万・E.....弱・反応性・
示・10mM EDTA・含・0.02%.....溶液
(.....社製)処理 A431 細胞抽出液中.....
・物.....反応.....SDS.....
.....電気泳動法及.....法.....
・確認・た.....

[0020]

SHE3-10.....HECD-1・SHE78-7・同様・
10mM CaCl_2 ・含・0.02%.....溶液処理
A431 細胞抽出液中・分子量約10万・E.....
.....強・反応性・示・10mM EDTA・含
・0.02%.....溶液処理 A431 細胞抽出液
中.....物.....反応.....SDS.....
.....電気泳動法及.....
.....法.....確認・た.....

・た SHE3-10・分子量約8万・胎盤由来
可溶性E.....対・SHE13-6・同等・
反応性・有す.....ELISA・法.....確認・
た.....

更・SHE3-10・そ表面・十分・洗淨・た
A431 細胞表面・強・反応性・示・SHE13-6
・弱・反応性・示す.....
.....法.....確認・た.....

[0021]

.....結果.....SHE13-6・SHE3-10.....
HECD-1・SHE78-7・同様・標準E.....
反応性・有す.....抗体.....
.....確認・得た.....

該 SHE13-6 産生.....Hybridoma
SHE13-6・表示・命名・工業技術院生命工

standard Ecadherin due to suitable amount SD
Spolyacrylamide-gel electrophoresis, insame way as HEC
D-1・SHE78-7 by western blot method which uses those
which are copied to electrical in nitrocellulose film.

[0019]

In addition, occasion where cultured cell etc which reveals
cadherin as property which is in common to cadherin family,
is dispersed with the trypsin (EC 3.4.21.4) or other protease,
under 2 - 10 mM calcium ion coexisting cadherin is
notdisassembled and maintains shape of that way molecule
and remains, but Under 2 - 10 mM EDTA coexisting under or
other calcium ion absent condition cadherin is disassembled
and phenomena that is verified, molecule disappears, (M.
bamboo I jp8 (M.Takeichi) and others, rope mentha
jp11・biology (development al biology), 8 th 7 volumes, third
40-350 page (1981)).

SHE1 3- 6, it showed reactivity which is weak in Ecadherin of
molecular weight approximately 100,000 in 0.02% trypsin
(EC 3.4.21.4) solution (furo supplied) process A 4 31
cell extracted liquid which includes 10 mM CaCl_2 it does not
react, by SD Spolyacrylamide-gel electrophoresis or western
blot method itverified even in what ones in 0.02% trypsin (EC
3.4.21.4) solution (furo supplied) process A 4 31 cell
extracted liquid which includes 10 mM EDTA.

[0020]

SHE3- 10, it showed reactivity which is strong in Ecadherin
of molecular weight approximately 100,000 in 0.02% trypsin
(EC 3.4.21.4) solution treatment A4 31 cell extracted liquid
which includes 10 mM CaCl_2 in same way as HEC D-1・
SHE78-7 it does not react, by SD Spolyacrylamide-gel
electrophoresis or western blot method it verified even in
what ones in 0.02% trypsin (EC 3.4.21.4) solution treatment
A4 31 cell extracted liquid which includes 10 mM EDTA.

In addition, SHE3- 10 it possesses SHE1 3- 6 and identical
reactivity vis-a-vis human placenta derivative solubility
Ecadherin of molecular weight approximately 80,000,
youverified by ELISA method.

Furthermore, SHE3- 10 shows reactivity which is strong in
A4 31 cell surface which washed surface in fully SHE1 3- 6
shows weak reactivity you verified by flow site tri-
method.

[0021]

From these results, SHE1 3- 6・SHE3- 10 when it is a mouse
monoclonal antibody which possesses the reactivity in same
way as HEC D-1・SHE78-7 in standard Ecadherin, acquired
conclusive evidence.

said SHE1 3- 6 production hybridoma Hybridoma SHE1 3- 6
and it indicated, designated, the deposit did in Agency of

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学工業技術研究所・寄託・た(FERM P-13389)・

・た該 SHE3-10 産生・
Hybridoma SHE3-10・表示・命名・工業技術
院生命工学工業技術研究所・寄託・た(FERM
P-14144)・

[0022]・

(3)・
Hybridoma SHE13-6 (FERM P-13389)・
・(和光純薬社製)・め投与・た
Balb/c・10週令(日本・社製)腹腔内・
大量・増殖・投与後約10日経過・た時点
・腹水・採集・硫酸塩析法又・
・交換・一般・抗体精製
・用・方法・精製・行・精製
SHE13-6・得た・

SHE13-6・過・素酸法・キ・
・(社製)・標識・
た・

標識E・吸着・た96穴・
・固相・SHE3-10・SHE78-7・又・
HECD-1・吸着抗原・反応・
そ・依存的・前述・酵素標識 SHE13-6・
吸着抗原・結合・阻害・
ELISA 法・調査・た・両者・抗体間
・全・競合阻害性・見出・
・た・

す・SHE13-6・他・3種・抗体・す・
・SHE3-10・SHE78-7・及・HECD-1・標準
E・握・結合・競合・た・
・E・分子上 SHE3-10・SHE78-7・及
・HECD-1・認識部位・異・部位・認識
す・確認・た・

Hybridoma SHE3-10 (FERM P-14144)・調製
・た精製 SHE3-10・及・前出・SHE78-7・
・そ・同様・検討・行・自身
・除・他・3種・抗体・抗原結
合・関・競合・確認・た・

・SHE13-6・SHE3-10・SHE78-7・及・
HECD-1・E・分子上異・
・部位・認識す・確認・た・

[0023]・

(4)・EIA 系・構築

HECD-1・SHE13-6・そ・固相化抗体・酵

Industrial Science and Technology National Institute of
Bioscience and Human-Technology (FERM P-13389).

In addition, said SHE3-10 production hybridoma, Hybridoma
SHE3-10 and it indicated, designated deposit did in Agency
of Industrial Science and Technology National Institute of
Bioscience and Human-Technology (FERM P-14144).

[0022]

Verification Hybridoma SHE13-6 (FERM P-13389) of
specificity of (3) monoclonal antibody, with Balb/c mouse 10
week (Clea Japan Inc. (DB 69-073-1062) supplied)
intraperitoneal which prescribes pre stain (Wako Pure
Chemical Industries Ltd. (DB 69-059-8875) supplied)
beforehand multiplying in large scale, after prescribing with
time point which approximately 10 days; passage is
done you collected spleen from mouse, such as ammonium
sulfate salting-out is well used for antibody purification
generally or ion exchange chromatography you refined, with
method which acquired refining SHE13-6.

SHE13-6 labelling was done with peroxidase (Boehringer
Mannheim supplied) with periodic acid method.

Whether or not connection to adsorption antigen of
theaforementioned enzyme labelling SHE13-6 inhibition
designates SHE3-10・SHE78-7・or HEC D-1 as adsorption
antigen in dose dependent it reacts by, labelling Ecadherin
96-hole microplate where it adsorbs as solid phase, when you
investigated with the ELISA method, completely that kind of
competitive inhibition characteristic did not discover between
antibody of both.

namely, SHE13-6 did not compete to connection to standard
Ecadherin antigen of antibody・namely SHE3-10・
SHE78-7・or HEC D-1 of other 3 kinds, therefore the SHE3-
10・SHE78-7・on Ecadherin molecule and recognition site of
HEC D-1 site which differs is recognized verified.

It did respective similar examination Hybridoma SHE3-10
(FERM P-14144) from concerning refining SHE3-10・, and
depicted above SHE78-7 which are manufactured in each
case it does not compete it verified in regard to monoclonal
antibody and antigen connection of other 3 kinds which
exclude itself.

Depending, you recognize site where in each case top of the
Ecadherin molecule differs you verified SHE13-6・SHE3-
10・SHE78-7・and HEC D-1.

[0023]

Construction of (4) sandwich EIA system

sandwich EIA was constructed with HEC D-1 and SHE13-6

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素標識抗体 ELA . 構築 . た . as solidification antibody . enzyme labelled antibody respectively.

. . . PBS . 終濃度 1mg/ml . HEC D-1 . 含 .
溶液 . 調製 化 終濃度 0.1%
. 添加 . 固相抗体液 . 調製す . .

該固相抗体液 (0.1% .
. 含 . PBS) . . . 100 倍 . 希釈 .
た終濃度 10 μ g/ml . 抗体溶液 . 96 穴 . . .
. (. F-16 社) .
各穴 . 200 μ l . . . 添加 . 密封 . 4 deg C .
24 時間静置す . .

次 溶液 . 捨 キ . 液
(0.1% 含 . BSA/PBS) . . .
. . . 各穴 . 200 μ l . . . 添加 . 密封 . 37
deg C . 1 時間静置す . .

. PBS . 2 回洗淨 . . . 測定試料又 .
標準 E 800 400 200 100 50 .
0ng/ml 含有す 標準品溶液 各穴 .
100 μ l . . . 添加す . .

そ . . . 室温 . . 1 時間静置 . た後 . PBS .
. 各穴 . 3 回洗淨 キ
標識 . た SHE13-6 . 適度 . BSA/PBS . . 希釈
. 各穴 . 100 μ l . . . 添加す . .

更 . 室温 1 時間静置 . た後 . .
PBS 各穴 . 3 回洗淨す . .

. 各穴 . 溶液 . . . 取除き
. 2 塩酸塩錠 (. 社) 1 錠 . 基質
溶解液 (0.01% . 過酸化水素 . 含 酸緩
衝液 pH5.0) 10ml . 溶解 . (終濃度 1mg/ml) .
該基質溶液 各穴 . 100 μ l . . . 添
加 15 分間静置す . .

次 . 1N 硫酸溶液 各穴 . 100 μ l .
. . . 添加 . 酵素 . . . 発色反応 . 停止 . . . 速 .
. . . 96 穴 各穴 . 発色
. . . (. 社製) . . . 波長 492nm . 吸光計測 .
. . . 定量す . .

標準 E 0-800ng/ml . 標準品溶液 .
吸光度 . 表示濃度 検線 . 作製 . 各
測定試料液 . 吸光度 . 検線 . 用 体液
可溶性 E 濃度 . 算定す . .

代表的 . 検線 . 図 1 . 示 . た .

す 約 0-800ng/ml . . . 低濃度域 . 測定
可能 た .

First solution which includes HEC D-1 of final concentration 1 mg/ml with PBS is manufactured, sodium azide is added in order to become final concentration 0.1%, the solid phase antibody liquid is manufactured.

said solid phase antibody liquid 96-hole microplate it adds 200;mu l at a time antibody solution of final concentration 10 ;mu g/ml which with coating buffer (PBS which includes 0.1% sodium azide) is diluted in 100 times, to each hole of (module plate F-16 " jp10 " corporation), seals up and 24 hours standing does with 4 deg C.

Next, you throw away solution from plate, add 200;mu l at a time blocking liquid (BSA/PBS which includes 0.1% sodium azide) to each hole of plate, seal up and 1 hour standing dowith 37 deg C.

plate twice is washed with PBS, measurement sample or standard Ecadherin 800, 400 and 20 0, 1 00, 50 and 0 ng/ml standard article solution which is contained is added 100;mu l at a time to each hole of plate.

That way, with room temperature 1 hour standing after doing, thrice you wash the plate each hole with PBS, you dilute SHE1 3- 6 which peroxidase labelling is done moderately with BSA/PBS and add 100;mu l at a time to each hole of plate.

Furthermore after 1 hour standing doing that way with room temperature , plate each hole thrice is washed with PBS.

It removes solution well than each hole of plate, ortho phenylenediamine dihydrochloride pill (Sigma Chemical Co.) substrate dissolved liquid (citric acid buffer " pH 5.0 which includes 0.01% hydrogen peroxide) melts 1 pill in 10 ml and (final concentration 1 mg/ml), said matrix solution adds 100;mu l at a time to each hole of plate, 15 min standing does that way.

It adds 100;mu l at a time 1 N sulfuric acid solution to each hole of plate next and stops coloration reaction with enzyme , 96-hole colors each hole of the plate rapidly with plate reader (furo " supplied) with light absorption measurement of the wavelength 492 nm quantification.

measuring line is produced with with absorbance and indicator concentration of standard article solution of standard Ecadherin 0-800 ng/ml, body fluid solubility Ecadherin concentration is computed making use of measuring line from absorbance of each measurement sample liquid.

representative measuring line was shown in Figure 1.

It was a measurable with low concentration region, namely, approximately 0 - 800 ng/ml.

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・ ・ ・ SHE78-7 ・ SHE3-10 ・ そ ・ ・ ・ 固相化抗体
 体 ・ 酵素標識抗体 ・ ・ ・ 場合 ・ ・ ・ 上記 ・ 全 ・ 同
 様 ・ ・ ・ ・ ・ EIA ・ 構築す ・ ・ ・ ・ ・ 爾 ・ ・
 ・ た ・

【0024 ・

(5) 体液試料 ・ 測定

悪性腫瘍患者血清 51 例 ・ 健康成人血清 18 例 ・
 悪性腫瘍患者尿 21 例 ・ 健康成人尿 20 例 ・ 対
 象 ・ ・ ・ HEC D-1 ・ SHE13-6 ・ 組合 ・ ・ ・ ・ 実
 施例 1-(4) ・ 記載 ・ 方法 ・ ・ ・ 体液可溶性 ・ ・
 ・ ・ ・ 濃度 ・ 測定 ・ た ・

・ ・ ・ ・ 尿 ・ 随時尿 ・ た ・ め ・ ・ ・ ・ ・
 ・ ・ ・ ・ ・ ・ ・ ・ ・ ・ ・ (和光純薬社製)キ
 ・ ・ ・ ・ 同時 ・ 測定 ・ ・ 尿中 ・ 体液可溶性 ・ ・
 ・ ・ ・ ・ 量 ・ 尿g ・ ・ ・ ・ ・ 当 ・ ・ ・ 体液可溶性
 E ・ ・ ・ ・ ・ (値g/g ・ ・ ・ ・ ・) ・ ・ ・ 算出
 た ・

・ た ・ 試料 ・ 血清 ・ 尿 ・ ・ ・ ・ BSA/PBS ・ ・
 100 倍 ・ 希釈 ・ 測定 ・ た ・

各血清中 ・ 体液可溶性 E ・ ・ ・ ・ 濃度 ・ 分布
 ・ 図 2 ・ ・ 各尿中 ・ 体液可溶性 E ・ ・ ・ ・ 値
 ・ 分布 ・ 図 3 ・ 示す ・

図 2 ・ 示す ・ ・ ・ ・ 血清中 ・ 体液可溶性 ・ ・ ・
 ・ ・ ・ 濃度 ・ 正常値上限 ・ $3 \mu\text{g/ml}$ ・ 設定 ・ た場
 合 ・ 悪性腫瘍陽性率 ・ 51%(26 例陽性/全 51
 例中) ・ 健康成人 ・ 全例陰性 ・ ・ ・ た 0 例陽性/
 全 18 例中) ・

・ た 図 3 ・ 示す ・ ・ ・ ・ 尿中 ・ 体液可溶性 ・ ・
 ・ ・ ・ ・ 値 正常値上限 ・ 11.7mg/g ・ ・ ・ ・
 ・ ・ 認定 ・ た場合 ・ 悪性腫瘍陽性率 ・ 57%(12
 例陽性/全 21 例中) ・ 健康成人 ・ 全例陰性 ・ ・
 ・ た 0 例陽性/全 20 例中) ・

・ ・ ・ ・ ・ 血清中 ・ 尿中 ・ 体液可溶性 ・ ・ ・
 ・ ・ ・ 量 正常値 ・ 比較す ・ ・ ・ ・ ・ 悪性腫瘍
 ・ 検出 ・ 可能 ・ ・ ・ ・ た ・

【0025 ・

実施例 2

キ 築

表 1 ・ 示す ・ ・ ・ ・ 体液可溶性 ・ ・ ・ ・ 測定
 用 EIA キ 築 ・ た ・

・ ・ ・ ・ 酵素標識抗体 ・ 精製 ・ た SHE13-6 ・
 ・ ・ ・ キ ・ ・ ・ ・ 酵素標識 ・ 核標識抗体 ・

Furthermore, sandwich EIA is constructed was possible completely in the same way as description above even with when SHE78-7 and the SHE3-10 are designated as solidification antibody ・ enzyme labelled antibody respectively.

【0024】

Measurement of (5) body fluid specimen

body fluid solubility Ecadherin concentration was measured due to method which with combination of the HEC D-1 and SHE13-6 is stated in Working Example 1-(4) with malignant tumor patient blood serum 51 example, healthy adult blood serum 18 example, malignant tumor patient urine 21 example and healthy adult urine 20 example as object.

urine among these on occasion because of urine, measured the creatine in urine simultaneously due to creatine ・ test ・ Wako (Wako Pure Chemical Industries Ltd. (DB 69-059-8875) supplied) kit, calculated body fluid solubility Ecadherin quantity in urine body fluid solubility Ecadherin value per urine g ・ creatine (mg/g ・ creatine) as.

In addition, specimen with BSA/PBS diluted blood serum ・ urine in each case in 100 times and measured.

distribution of body fluid solubility Ecadherin concentration in each blood serum in Figure 2, distribution of body fluid solubility Ecadherin value in each urine is shown in Figure 3.

As shown in Figure 2, as for malignant tumor positive ratio when normal value upper limit of body fluid solubility Ecadherin concentration in blood serum is set $3 \mu\text{g/ml}$ 51% (In 26 example positive/ all 51 examples), as for healthy adult they were all the example negative, (In 0 example positive/ all 18 examples).

In addition, as shown in Figure 3, as for malignant tumor positive ratio when normal value upper limit of body fluid solubility Ecadherin value in urine is recognized $11.7 \text{ mg/g} \cdot \text{creatinine}$ 57% (In 12 example positive/ all 21 examples), as for the healthy adult they were all example negative, (In 0 example positive/ all 20 examples).

This way detection of malignant tumor was possible by comparing body fluid solubility Ecadherin quantity in blood serum and in urine with normal value.

【0025】

Working Example 2

Construction of kit

As shown in Table 1, body fluid solubility Ecadherin measurement EIA kit was constructed.

Among these, SHE13-6 which was refined enzyme labelling it did enzyme labelled antibody with peroxidase, with

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終濃度 1mg/ml IgG 含・溶液・BSA/PBS・
1000 倍・希釈・瓶・1 本・
き 11ml 封入・そ・凍結乾燥・た・調
製・た・

検体希釈液・0.1%・化・含・
BSA/PBS・0.22 μ m 口径・
過滅菌・た・調製・た・

標準品・実施例 1-(1)・調製・た標準 E・
0.1%・化・含・BSA/PBS
希釈・800ng/ml 終濃度・溶液 作製
標準品溶液・瓶 1 本・
1ml 封入・そ・凍結乾燥・た・調製・
た・

そ 他・実施例 1-(4)・記載・使用・
た・

[0026]

[表 1]

BSA/PBS it diluted solution which said labelled antibody
final concentration 1 mg/ml IgG is included in 1000 times, 11
ml it enclosed in glass vial bottle concerning 1, it
manufactured those which lyophilizing are done that way.

test agent diluent BSA/PBS which includes 0.1% sodium
azide manufactured those which filtration sterilization are
done with membrane filter of 0.22 μ m aperture.

standard Ecadherin which is manufactured with Working
Example 1- (1) it diluted standard article with BSA/PBS
which includes 0.1% sodium azide and it produced solution
which becomes 800 ng/ml final concentration, 1 ml enclosed
this standard article solution in glass vial bottle 1,
it manufactured those which lyophilizing are done that way.

Other things used those which are stated in Working Example
1- (4).

[0026]

[Table 1]

・内容物・	容量・		・本	数・
<contents>	volume>		< Book	Number >
固相抗体液	・ μ ・	X	・	・
solid phase antibody liquid	20; μ l	X	1	vial
・	・ ml	X	・	・
coating buffer	11 ml	X	2	vial
・ キ ・ 液	・ ml	X	・	・
blocking liquid	11 ml	X	2	vial
酵素標識抗体 凍結乾燥品	・ ml 用	X	・	・
enzyme labelled antibody (lyophilized product)	11 ml	X	1	vial
標準品 凍結乾燥品	・ ml 用	X	・	・
standard article (lyophilized product)	1 ml	X	1	vial
検体希釈液	・ ml	X	・	・
test agent diluent	11 ml	X	2	vial

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.....錠			・	錠		
ortho phenylenediamine pill			2	pill		
基質溶解液	・ ml	×	・		
substrate dissolved liquid	11 ml	X	2	vial		
・ 穴			・	枚		
96 -hole microplate			1			

【0027・

【0027】

{

[Effects of the Invention]

以上詳細・説明・た 本発明 迅速・簡便・体液中・体液可溶性E 測定方法・確立 新規・悪性腫瘍・検出方法及・キ た・

As above explained in detail, being quick with this invention, body fluid solubility Ecadherin assay in simple body fluid was established, detection method and kit of novel malignant tumor were offered.

【図面・簡単・説明・

[Brief Explanation of the Drawing(s)]

【図・

[Figure 1]

実施例 1-(4) EIA 系・使用す・検量・示す

It is a graph which shows measuring line which is used with sandwich ELA system in Working Example 1-(4).

【図・

[Figure 2]

本発明・方法・測定・た 血清中・体液可溶性 E 濃度・測定結果・示す図

It is a figure which shows measurement result of body fluid solubility Ecadherin concentration in blood serum which was measured with method of this invention.

【図・

[Figure 3]

本発明・方法・測定・た 尿中・体液可溶性 値・測定結果・示す図

It is a figure which shows measurement result of body fluid solubility Ecadherin value in urine which was measured with method of this invention.

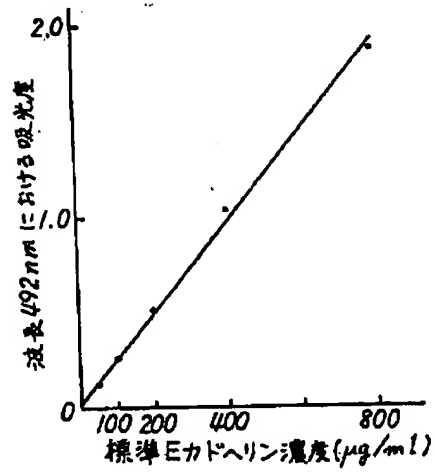
Drawings

【図・

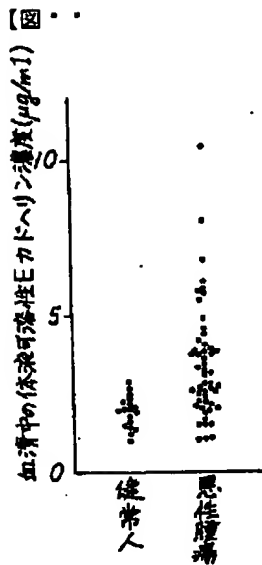
[Figure 1]

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[Figure 2]



[Figure 3]

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